AD NUMBER

AD469244

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; May 1965. Other requests shall be referred to Director, Arctic Aeromedical Laboratory, Ft Wainwright, Ak

AUTHORITY

OAR ltr, 18 Nov 1965

SECURITY MARKING

The classified or limited status of this report applies to each page, unless otherwise marked.
Separate page printouts MUST be marked accordingly.

THIS DOCUMENT CONTAINS INFORMATION AFFECTING THE NATIONAL DEFENSE OF THE UNITED STATES WITHIN THE MEANING OF THE ESPIONAGE LAWS, TITLE 18, U.S.C., SECTIONS 793 AND 794. THE TRANSMISSION OR THE REVELATION OF ITS CONTENTS IN ANY MANNER TO AN UNAUTHORIZED PERSON IS PROHIBITED BY LAW.

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

AAL-TR-64-27

IRON METABOLISM AND TRYPTOPHAN PYRROLASE ACTIVITY IN ENDOTOXIN-POISONED AND CORTISONE-PROTECTED MICE

George N. Eaves and L. Joe Berry

May 1965

ARCTIC AEROMEDICAL LABORATORY

AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
FORT WAINWRIGHT, ALASKA

IRON METABOLISM AND TRYPTOPHAN PYRROLASE ACTIVITY IN ENDOTOXIN-POISONED AND CORTISONE-PROTECTED MICE

George N. Eaves and L. Joe Berry

FOREWORD

This is the sixth quarterly report on contract AF41(609)-1764 (Project 8241, Task 824101) with the Department of Biology, Bryn Mawr College, Bryn Mawr, Pa. Research covered in the report was done from 1 June 1964-31 August, 1964, and the report was submitted 12 October 1964. Air Force program monitor is Robert Becker, ALRA, Arctic Aeromedical Laboratory.

This technical report has been reviewed and is approved.

PUBLICATION REVIEW

Horace F. Drury

HORACE F. DRURY
Director of Research

ABSTRACT

The hypoferremia which follows the intraperitoneal injection of mice with heat-killed Salmonella typhimurium is not mitigated by protection with cortisone given simultaneously. A single injection of cortisone causes a transient hyperferremia. None of these changes in concentration of plasma iron occurs when mice are maintained in a 5° C environment during experimental treatment.

The alteration in iron metabolism during endointoxication does not seem to affect the availability of the iron-porphyrin coenzyme of tryptophan pyrrolase. An initial response of mice to injections of endotoxin or cortisone or both is a translocation of hematin, and the availability of hematin permits an increase in tryptophan pyrrolase activity before the stress-mediated induction of the enzyme becomes apparent. The increase in available activator, with the subsequent decrease in amount of normally inactive apoenzyme, occurs at the same rate and to the same extent in mice housed at 5°C and 25° C. It is suggested that the increased demand for an iron-containing substance in the liver during stress contributes to the accompanying hypoferremia.

I

INTRODUCTION

The decrease in activity of liver tryptophan pyrrolase which accompanies endotoxic poisoning in mice has been described by Berry and Smythe (1). Since it has been demonstrated by Kampschmidt and Arrendondo (2) that endotoxin alters iron metabolism in rats, it was considered possible that the decrease in tryptophan pyrrolase activity was associated with limitations in availability of the iron-porphyrin coenzyme. Therefore, it was considered relevant to investigate the relationship between changes in iron metabolism and the availability to tryptophan pyrrolase of its hematin activator during endointoxication in mice. Since it was known that adding excess hematin to unfractionated liver homogenates of mice increases tryptophan pyrrolase activity (3), earlier investigations could be extended to include determinations of the degree of saturation of the enzyme with respect to its cofactor, as well as actual amounts of apoenzyme formed during endointoxication and its alleviation by certain pharmacological agents. In addition, since the toxic manifestations of endotoxin appear to be different in animals stressed by cold (4), these investigations have included preliminary studies on iron metabolism and tryptophan pyrrolase activity during periods of stress mediated by environmental temperature.

II

METHODS

Mice. Female Swiss-Webster mice (Dierolf Farms, Boyertown, Pa.) weighing 23-25 gm were used in all experiments. They were housed 10 per cage, with pine shavings as bedding, in an animal room held at $25^{\circ} \pm 2^{\circ}$ C, and given water and D and G pathogen-free mouse food (Price-Wilhoite Co., Frederick, Md.) ad libitum until the beginning of an experiment. Tetracycline antibiotics (Polyotic, American Cyanamide Co., Princeton, N. J.) were added to the drinking water for the first two days after arrival of the mice from the dealer. The drinking water was free of antibiotics at least one week before the mice were used experimentally.

Experimental infection and treatment. Endotoxin was in the form of a saline suspension of heat-killed Salmonella typhimurium, strain SR-11, as described previously (1). The dry weight of the suspension was 5.7 mg per ml. One LD_{50} (9.1 μg at 5° C, 0.91 mg at 25° C, and 3.8 μg at 37° C) was given, unless noted otherwise.

Substances to be injected were diluted with nonpyrogenic saline (Baxter Laboratories, Morton Grove, Ill.). All injections were given in a volume of 0.5 ml. Cortisone acetate (United Research Laboratories, Inc., Philadelphia, Pa.) and adenosine triphosphate (ATP) (Sigma Chemical Co., St. Louis, Mo.) were given in 5 mg amounts. Cortisone was given subcutaneously; all other injections were given intraperitoneally.

The mice were deprived of food and water from the beginning of experimental treatment.

Environmental studies. Mice to be exposed to an environmental temperature different from that in which they were routinely maintained were placed in a Modu-Lab Room (Labline, Inc., Chicago, Ill.) at the desired temperature (5° C or 37° C) immediately after injection. The relative humidity was maintained at 30%. The mice were protected from drafts by covering the draft-exposed area of the incubator shelves with plastic sheeting.

Determination of plasma iron and copper. Plasma iron and copper were determined by the method of Landers and Zak (5), with the following modifications. Each determination was performed on pooled plasma from five identically treated mice, the blood of which was obtained by supraorbital bleeding. After heating the plasma with N-HCl, 1.0 ml of 10% trichloroacetic acid was added and the resulting precipitate removed by centrifugation at 9000 X g for 15 minutes.

Determination of liver iron and copper. Immediately after removal of blood by supraorbital bleeding, the liver was removed to the surface of dry ice and subsequently stored in a freezer. Aliquots (0.1 gm) from five livers were pooled and homogenized in water with a Teflon and Pyrex homogenizer. Tissue adhering to the homogenizer was washed into the homogenizing tube with 5 ml water. Of the resulting suspension, 5 ml was transferred to a polypropylene centrifuge tube and 2.5 ml N-HCl added and mixed. An aliquot of the remaining homogenate was used for the determination of dry weight. The homogenate-HCl mixture was heated in a 95° C water bath for 10 minutes and subsequently cooled in tap water. The resulting precipitate was removed by centrifugation at 270 X g for 15 minutes. A 1.5 ml aliquot of the supernatant fluid was removed to a polypropylene centrifuge tube and treated as for the determination of plasma iron and copper after the plasma had been heated with N-HCl.

Distilled water which had been passed through a research model de-ionizer (Illinois Water Treatment Co., Rockford, Ill.) was used in all determinations of iron and copper.

Determination of tryptophan pyrrolase activity. Tryptophan pyrrolase was determined by the method of Knox and Auerbach (6) as modified for mice by Berry and Smythe (1). Further modifications were as follows. Each liver

homogenate was assayed for tryptophan pyrrolase activity with and without the addition of 5.0 μM hematin, which was made immediately before use by dissolving twice-crystallized bovine hemin (Sigma Chemical Co.) in dilute sodium hydroxide. The flasks containing the reaction mixture were incubated at 38° C in a table model water bath shaker (Eberbach Corp., Ann Arbor, Mich.) equipped with a hood for controlled atmosphere. Oxygen was added during the first 5 minutes of incubation, after which the outlets of the hood were opened to room environment. The neutralized filtrate was clarified by centrifugation at 20,000 X g for 15 minutes.

The activity of tryptophan pyrrolase without the addition of excess hematin is considered to be indicative of in vivo holoenzyme activity and is therefore referred to as the "active" apoenzyme. The addition of excess hematin reveals the additional amount of apoenzyme which is potentially active and is therefore referred to as "total" apoenzyme. The difference in activity with and without the addition of excess activator is considered to be a measure of the amount of "inactive" apoenzyme in the liver homogenate.

III

RESULTS

Plasma iron and copper. The changes in plasma iron during endointoxication and treatment with cortisone are shown in Figure 1. Plasma iron is lost rapidly during the first 6 hours after administration of one LD_{50} of heat-killed Salmonella typhimurium. This decrease in iron concentration becomes significant during the first 2 hours of endotoxic poisoning. In contrast, there is a rise in plasma iron during the first 4 hours after injection of cortisone. The administration of cortisone concurrently with endotoxin fails to prevent the loss of plasma iron associated with endointoxication.

Copper, which catalyzes the formation of heme from protoporphyrin and iron, increases in the plasma with infections which result in hypoferremia (7). As shown in Table I, a significant decrease in copper during the first 4 hours is followed by an increase at 6 hours. The increase in copper is less in mice given endotoxin; all other groups show a more pronounced hypercupremia.

The concurrent injection of 5 mg of ATP protects against the lethal effects of endotoxin. Only one mouse out of ten given 2 X LD_{50} (1.82 mg) of endotoxin survived for 24 hours, in contrast to seven survivors out of ten which were given ATP concurrently: $P = 0.022$ by the rank order test of Wilcoxon (8). As shown in Table II, ATP fails to prevent the loss of plasma iron associated with endointoxication.

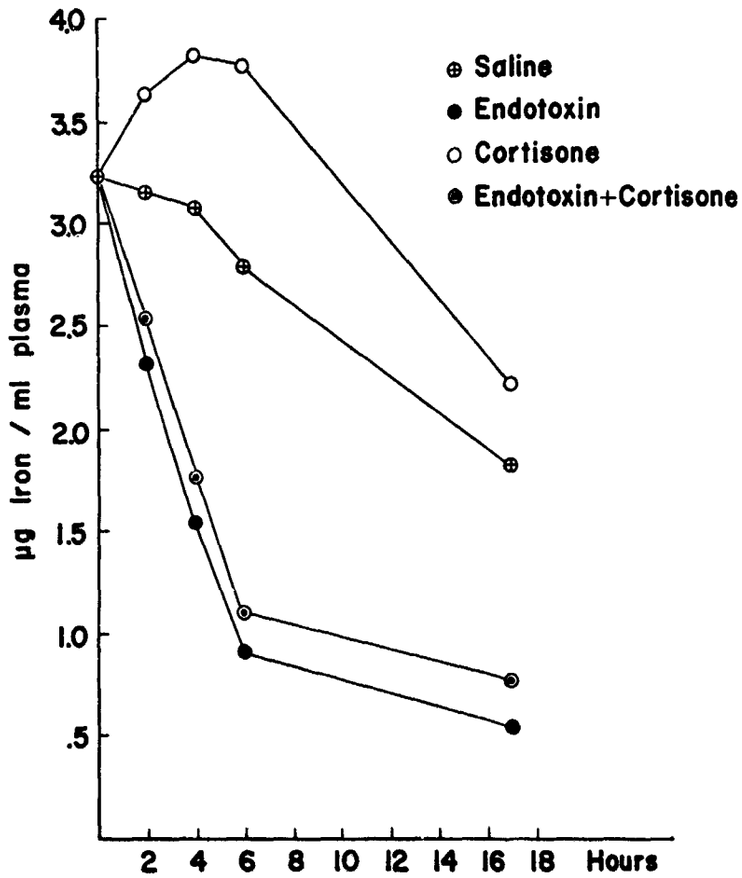

FIGURE 1

Effect of endotoxin and cortisone, together and separately, on plasma iron concentration of mice.

TABLE I

EFFECT OF ENDOTOXIN ON PLASMA COPPER

Experimental Treatment	Hours after Experimental Treatment				
	0	2	4	6	17
Saline, 0.5 ml	1.15 ± 0.07* (8)**	0.86 ± 0.09 (9)	0.88 ± 0.07 (7)	1.15 ± 0.04 (7)	1.05 ± 0.05 (9)
Endotoxin, LD_{50}		0.88 ± 0.12 (8)	0.72 ± 0.03 (8)	1.01 ± 0.06 (8)	1.12 ± 0.04 (9)
Cortisone, 5 mg		1.00 ± 0.05 (7)	0.85 ± 0.05 (8)	1.33 ± 0.04 (8)	1.14 ± 0.05 (7)
Cortisone and Endotoxin		0.87 ± 0.07 (7)	0.74 ± 0.02 (8)	1.21 ± 0.04 (7)	1.53 ± 0.07 (7)

* Mean ± standard error

** In this and all succeeding tables, numbers in parentheses indicate numbers of determinations made.

TABLE II

EFFECT OF ATP ON PLASMA IRON

4 Hours after Injection of	Iron (μg / ml plasma)
ATP, 5 mg	2.3* (2)**
Saline, 0.5 ml	3.1 (7)
Endotoxin, LD_{50} (0.91 mg)	1.5 (8)
Endotoxin and ATP	1.5 (3)

* Mean
** Each determination is pool of five mice.

Mice exposed to 5° C immediately following experimental treatment show no changes in either plasma iron (Table III) or copper levels during the first 4 hours, which is the time when the lethal manifestations of endotoxin poisoning become apparent. (Number of survivors/number injected: saline, 41/44; endotoxin, 38/54; cortisone, 39/40; cortisone and endotoxin, 37/41.)

Liver iron and copper. As shown in Table IV, significant differences in iron content could not be detected in livers of normal and endointoxicated mice by the method employed. However, there is a significant decrease in liver copper in poisoned mice.

Tryptophan pyrrolase activity. Tables V and VI show the active, total and inactive tryptophan pyrrolase activity in liver homogenates from mice which have been injected with saline, endotoxin, cortisone and both endotoxin and cortisone. The injection of saline alone results in an increase in the total amount of apoenzyme 17 hours after injection; there is little change in amount of active tryptophan pyrrolase. The increase in total tryptophan pyrrolase of saline-injected mice is small, however, as compared to uninjected controls. At the end of 17 hours, mice given endotoxin have only half as much active and total tryptophan pyrrolase as mice given saline. Similarly, the induction of tryptophan pyrrolase by cortisone is limited by endotoxin (Table VI). In mice given concurrent injections of cortisone

TABLE III

EFFECT OF ENDOTOXIN ON PLASMA IRON FOLLOWING EXPOSURE OF MICE TO 5° C and 25° C

4 Hours after Injection of	Iron (μg/ml plasma)	
	5° C	25° C
Saline, 0.5 ml	2.7 ± 0.2* (7)	3.1 ± 0.5 (7)
Endotoxin, LD_{50}**	2.6 ± 0.1 (7)	1.5 ± 0.4 (8)
Cortisone, 5 mg	2.8 ± 0.2 (7)	3.8 ± 0.3 (8)
Cortisone and endotoxin	2.6 ± 0.1 (7)	1.8 ± 0.4 (8)

* Mean ± standard error
** 5° LD_{50} (9.1 μg), 25° LD_{50} (0.91 mg)

TABLE IV

EFFECT OF ENDOTOXIN ON LIVER IRON AND COPPER

17 Hours after Injection of	Iron (μg/gm liver)	Copper (μg/gm liver)
Saline, 0.5 ml	141.5 ± 16.7* (4)**	35.0 ± 1.7 (4)
Endotoxin, LD_{50} (0.91 mg)	166.2 ± 12.1 (4) N.S.***	27.5 ± 0.9 (4) 0.02 <P> 0.01****

* Mean ± standard error
** (Each determination is pool of five mice.)
*** Not statistically significant
**** "t" test of significance

TABLE V

EFFECT OF ENDOTOXIN ON TRYPTOPHAN PYRROLASE ACTIVITY OF MICE

Hours after Injection	Activity of Tryptophan Pyrrolase Apoenzyme (μM kynurenine/gm liver/hr)					
	Control		Saline (0.5 ml)		Endotoxin (LD_{50})	
	Active	Total	Active	Total	Active	Total
0	12.5 ± 0.7* (8)	26.7 ± 2.0 114%**				
2	10.1 ± 1.0 (8)	20.8 ± 2.1 106%	12.8 ± 1.0 (8)	25.0 ± 2.0 95%	17.8 ± 1.2 (8)	24.5 ± 1.7 38%
4	10.6 ± 0.9 (8)	26.3 ± 2.5 148%	11.8 ± 1.1 (10)	18.2 ± 1.9 54%	11.7 ± 1.6 (10)	15.2 ± 2.2 30%
6	7.9 ± 0.8 (8)	16.9 ± 1.9 114%	16.0 ± 1.3 (10)	31.8 ± 2.3 99%	18.4 ± 1.2 (10)	24.4 ± 1.9 33%
17	15.4 ± 1.2 (8)	32.5 ± 2.2 111%	18.3 ± 2.3 (6)	41.2 ± 4.0 125%	9.0 ± 1.1 (6)	19.1 ± 2.4 112%

* Mean ± standard error

** Percent increase

TABLE VI

EFFECT OF CORTISONE ON TRYPTOPHAN PYRROLASE ACTIVITY OF ENDOINTOXICATED MICE

Hours after Injection	Activity of Tryptophan Pyrrolase Apoenzyme (μM kynurenine/gm liver/hr)			
	Cortisone (5 mg)		Cortisone and Endotoxin (5 mg) (LD_{50})	
	Active	Total	Active	Total
2	16.2 ± 1.7* (8)	23.3 ± 2.0 44%**	15.2 ± 2.0 (8)	20.2 ± 1.7 33%
4	22.1 ± 2.3 (10)	28.8 ± 3.7 30%	16.8 ± 1.8 (10)	19.5 ± 2.1 16%
6	44.2 ± 3.6 (10)	71.1 ± 3.8 61%	27.1 ± 3.5 (10)	36.6 ± 5.3 16%
17	31.3 ± 1.8 (6)	74.1 ± 5.4 137%	13.4 ± 1.1 (6)	34.8 ± 4.0 160%

* Mean ± standard error
** Percent increase

and endotoxin, the amount of tryptophan pyrrolase increases significantly during the first 6 hours after injection; but, again, after 17 hours there is only half as much active and total tryptophan pyrrolase as in mice given cortisone only. Tables V and VI also show the percent increase in activity when excess activator is added to the homogenate.

The increase in active apoenzyme of endointoxicated mice at 2 hours (Table V), without an accompanying change in amount of total apoenzyme, demonstrates an increased availability of intracellular hematin to the existing apoenzyme. Only a significant change in active apoenzyme level without a concomitant increase in total apoenzyme can be interpreted as indicating changes in coenzyme levels. Therefore, conclusions relating availability of coenzyme to the change in enzyme activity with addition of excess activator cannot be made once changes in both active and total apoenzyme begin to occur; i.e., after 2 hours. However, proportional considerations can be obtained by determining the percent of the total tryptophan pyrrolase activity

which is inactive during experimental treatment. The results of these calculations are shown in Tables VII and X. It can be seen that merely injecting the animals with saline ultimately results in a change from the normal proportion of apoenzyme and holoenzyme. This, however, occurs later than in animals subjected to the superimposed stresses of endotoxin and/or cortisone injections. The change in percent of inactive enzyme during experimental treatment is transient; the rapidity with which it appears, as well as the duration of its persistence, seems to be related to the magnitude of stress.

TABLE VII

EFFECT OF EXPERIMENTAL TREATMENT ON ACTIVATION OF TRYPTOPHAN PYRROLASE

Hours after Injection	Percent of Total Tryptophan Pyrrolase Which Is Inactive				
	Control	Saline (0.5 ml)	Endotoxin (LD_{50})	Cortisone (5 mg)	Cortisone and Endotoxin
0	52				
2	51	49	27	30	25
4	60	35	23	23	14
6	53	50	25	38	26
17	53	56	53	58	61

Determination of tryptophan pyrrolase activity in livers of mice which were maintained at 5° C immediately following experimental treatment demonstrates that the stress of cold alone results in increased levels of tryptophan pyrrolase (Table VIII). This stress-mediated induction is not prevented by endotoxin; however, the rate at which it occurs is slower in poisoned mice. As shown in Tables VIII and IX, there are no significant differences in levels of active enzyme or levels of inactive enzyme among the experimental groups at 6 hours, the time at which the lethal effects of endotoxin are demonstrable. Cortisone alone stimulates the induction of tryptophan pyrrolase in cold-exposed mice to a level higher than in mice given saline; however, endotoxin does not inhibit induction as it does in mice kept at 25° C (Table IX). There are no significant differences in tryptophan pyrrolase activity of cold-exposed mice given cortisone alone or with endotoxin.

In addition, the changes in levels of inactive apoenzyme following experimental treatment are the same in mice exposed to 5° C as those kept at 25° C (Table X).

TABLE VIII

EFFECT OF ENDOTOXIN ON TRYPTOPHAN PYRROLASE ACTIVITY OF MICE EXPOSED TO 5° C

Hours after Injection	Activity of Tryptophan Pyrrolase Apoenzyme (μM kynurenine/gm liver/hr)			
	Saline (0.5 ml)		Endotoxin (LD_{50})	
	Active	Total	Active	Total
2	15.1 ± 1.8* (4)	22.4 ± 2.2 48%**	12.7 ± 1.8 (4)	17.3 ± 3.1 36%
4	17.9 ± 1.6 (6)	30.4 ± 3.8 70%	15.0 ± 3.4 (6)	20.9 ± 5.1 39%
6	29.2 ± 4.5 (4)	43.4 ± 8.2 49%	24.4 ± 2.9 (8)	34.8 ± 7.0 43%

* Mean ± standard error
** Percent increase

Preliminary experiments on mice exposed to a 37° C environment indicate that tryptophan pyrrolase induction is simulated by heat stress; however, this induction is inhibited in endotoxin-poisoned mice. Table XI shows a comparison between amounts of total apoenzyme in mice maintained at three different environmental temperatures used in these studies. After 6 hours at 5° C and 25° C there is no significant difference between the total amount of enzyme activity in endointoxicated mice and their respective controls; however, at 37° C endotoxin has inhibited induction of the enzyme.

Table XII shows the activity of tryptophan pyrrolase in mice protected from the lethal effects of endotoxin by ATP. There are no differences between either the active apoenzyme or the total enzyme of mice 17 hours after injections of endotoxin alone or with ATP.

TABLE IX

EFFECT OF CORTISONE ON TRYPTOPHAN PYRROLASE ACTIVITY ON ENDOINTOXICATED MICE EXPOSED TO 5° C

Hours after Injection	Activity of Tryptophan Pyrrolase Apoenzyme (μM kynurenine/gm liver/hr)			
	Cortisone (5 mg)		Cortisone and Endotoxin (5 mg) (LD_{50})	
	Active	Total	Active	Total
2	12.2 ± 1.5* (4)	17.8 ± 3.0 47%**	10.3 ± 1.5 (4)	14.9 ± 3.1 46%
4	22.4 ± 2.5 (6)	34.5 ± 4.5 54%	13.9 ± 2.9 (5)	18.6 ± 3.1 34%
6	39.3 ± 4.2 (4)	68.0 ± 5.1 73%	36.9 ± 9.1 (2)	59.8 ± 18.4 62%

* Mean ± standard error

** Percent increase

TABLE X

EFFECT OF EXPERIMENTAL TREATMENT ON ACTIVATION OF TRYPTOPHAN PYRROLASE OF MICE EXPOSED TO 5° C

Hours after Injection	Percent of Total Tryptophan Pyrrolase Which is Inactive			
	Saline (0.5 ml)	Endotoxin (LD_{50})	Cortisone (5 mg)	Cortisone and Endotoxin
2	48	27	31	31
4	41	28	35	25
6	33	33	42	38

TABLE XI

EFFECT OF ENDOTOXIN ON TRYPTOPHAN PYRROLASE ACTIVITY OF MICE EXPOSED TO 5°, 25° and 37° C

6 Hours after Injection of	Tryptophan Pyrrolase Activity (Total Apoenzyme) (μM kynurenine/gm liver/hr)		
	5° C	25° C	37° C
Saline, 0.5 ml	43.4 ± 8.2* (4)	31.8 ± 2.3 (10)	29.3 ± 10.6 (5)
Endotoxin**	34.8 ± 7.0 (8) N.S.***	24.4 ± 1.9 (10) N.S.	14.6 ± 2.2 (5) 0.02<P>0.01****

* Mean ± standard error
** 5° LD_{50} (9.1 μg), 25° LD_{50} (0.91 mg), 37° LD_{50} (3.8 μg)
*** N.S. = not statistically significant
**** "t" test of significance

TABLE XII

EFFECT OF ATP ON TRYPTOPHAN PYRROLASE ACTIVITY IN ENDOINTOXICATED MICE

17 Hours after Simultaneous Injections of	Tryptophan Pyrrolase Activity (μM kynurenine/gm liver/hr)	
	Active Apoenzyme	Total Apoenzyme
ATP (5 mg) and saline (0.5 ml)	21.2* (2)	50.3
Endotoxin (LD_{50}) and saline	13.7 (2)	25.2
Endotoxin and ATP	10.5 (4)	21.0

* Mean

IV

DISCUSSION

It has been known for some time that hypoferremia, accompanied by an increase in plasma copper, develops very early in association with infection (7). Recently, Kampschmidt and Arrendondo (2) demonstrated a rapid decrease in the plasma iron concentration of rats which had been injected with endotoxin and suggested that endotoxin blocked the transport of iron from recently destroyed erythrocytes into the plasma. Freireich et al. (9) had proposed previously that inflammation interferred with the normal mechanism of catabolism and reutilization of erythrocyte iron, which resulted in a decrease of serum iron concentration. Our studies on the hypoferremia which accompanies endointoxication have been extended to include changes in plasma iron concentration which occur during protection of mice with cortisone. We have shown that a single, large dose of cortisone causes a rapid and transient hyperferremia in mice. Kumar et al. (10) and Cartwright et al. (11) have reported that blocking of the reticuloendothelial system (RES) resulted in a marked hyperferremia. It has also been shown by Heller (12) and Biozzi et al. (13) that cortisone reduces the normal phagocytic activity of the RES. Therefore, it could be anticipated that a hyperferremia would follow an injection of cortisone. Kumar et al. (10) suggested that some portion of the non-hemoglobin iron of blood is deviated to the reticuloendothelial (RE) cells under normal physiological conditions and that blocking these cells inteferred with their propensity to accept iron, which resulted in hyperferremia. It thus followed that if the reticuloendothelial cells utilize iron for their function, a greater requirement for iron would occur in conditions associated with increased RE cell activity. This interpretation is supported by the work of Cartwright et al. (11), which suggests that the hypoferremia accompanying sterile inflammation results from increased activity of the RES. Therefore, since the host' s defense against endotoxin depends, in part, upon a functional RES (14), it would seem logical to conclude that the hypoferremia associated with endointoxication is related to more demanding requirements for iron by the cells of the RES. It has, in fact, been shown that iron accumulates in the RES during mild infections in mice, a finding which was interpreted as indicating that infection stimulates the RES to store iron (15).

The concurrent injection of endotoxin and cortisone seems to represent a situation where the activity of the RES is depressed by the action of cortisone at the time when maximum activity would seem essential for the uptake of endotoxin. Biozzi et al. (13) had, in fact, observed that the stimulating effect of killed typhoid bacilli on the phagocytic activity of the RES was greatly reduced by treatment with cortisone. Therefore, one would expect that the presence of excess cortisone in an endointoxicated animal would prevent the decrease in plasma iron concentration. This, however, was not the case, for plasma iron decreased at the same rate in mice given cortisone and endotoxin as in mice given endotoxin only. In the experiments described

in this report, cortisone was given subcutaneously, and endotoxin was administered intraperitoneally. The latter route of injection assures faster absorption; therefore, endotoxin would presumably reach the cells of the RES before cortisone could exert its inhibitory effect. Such an explanation is supported by the finding that exposure to cold negates the changes in plasma iron concentration of mice given endotoxin, cortisone, or both. An initial response to the stress of cold would probably be an increased secretion of cortisone, which would decrease the activity of the RES and, consequently, its iron requirements. Without this increased demand for iron, the concentration of nonhemoglobin iron in the plasma would therefore remain unchanged.

The route of injection of endotoxin often determines the metabolic response of the host. For example, Cartwright et al. (16) found that a commercial typhoid (triple) vaccine given intramuscularly failed to produce hypoferremia in dogs. Biozzi et al. (13) found that a single large injection of heat-killed Salmonella typhi or endotoxin given intravenously caused a transient depression of the phagocytic activity of the RES, whereas the same amount of bacilli subcutaneously resulted in a stimulation of the RES.

The alteration in iron metabolism during endointoxication does not seem to include a limitation of the available iron-porphyrin activator, or coenzyme, of tryptophan pyrrolase. Indeed, there is an increase in available hematin following injection of endotoxin, cortisone or both. This immediate intracellular translocation of hematin may, however, represent an additional demand for iron, the replacement of which must ultimately find its source in the plasma reservoir and thus contribute to hypoferremia.

Kawachi (17) found no correlation between lowered plasma iron concentration and decreased tryptophan pyrrolase activity which occurred during the course of several weeks after inoculation of rats with Rhodamine fibrosarcoma. He concluded that the depressed tryptophan pyrrolase activity reflected a decreased amount of apoenzyme in the supernatant, but was affected somewhat by a slight decrease in amount of available activator.

The delayed occurrence of increased activation of tryptophan pyrrolase in mice injected with saline alone suggests that this phenomenon may be, in part, a response to the stress of injection; however, the magnitude of stress imposed by administration of a lethal toxin and/or a potent pharmacological agent results in a more immediate response. In no instance does there appear to be a limitation in availability of coenzyme. Even though the enzyme is normally only partially saturated with its coenzyme, there is apparently an available source of activator which is utilized in response to stress. The presence of excess apoenzyme, which can be readily activated following the translocation of hematin, would certainly represent a much more metabolically economical device for increasing enzyme activity without increasing the rate of its synthesis during physiological emergencies. When protein anabolism is possible, greater or more persistent, stress would

stimulate increased tryptophan pyrrolase synthesis through hormone-mediated induction and thus provide continuing assistance toward the maintenance of metabolic equilibrium.

The changes in activator levels which occur early in the course of experimental treatment are transient; within 17 hours after injection, the proportion of coenzyme to apoenzyme has returned to normal. In addition, these transitory changes seem to persist in relationship to the magnitude of stress, since only those animals given saline alone have normal activator levels as early as 6 hours after injection.

That the tryptophan pyrrolase of unfractionated liver homogenates of mice is normally only partially saturated with its coenzyme represents a contrast to the near-optimal amounts of activator available in rat liver homogenates (18). In the mouse, this may represent a seasonal variation, for the rather consistent increase in tryptophan pyrrolase activity with the addition of excess activator demonstrable during the winter months became unpredictable and extremely variable during warm weather. It is more likely, however, that this change in response represents inherent limitations in the assay of tryptophan pyrrolase in unfractionated liver homogenates of mice, since there is often no increase in activity with the addition of excess hematin in experiments where liver homogenates from identically treated animals show the expected, or often less, increase in activity. Further studies are being conducted on the assay of tryptophan pyrrolase in livers of mice, in hopes of eliminating this inexplicable inconsistency. Since the measure of available activator concentration is, by the existing assay, an indirect one, it limits the interpretation of data to that of trends, rather than facts, and serves as a guide for continuing investigations.

Mice exposed to cold immediately after injection of endotoxin are capable of inducing tryptophan pyrrolase. As suggested by the negation of plasma iron changes under identical conditions, this lack of depression of tryptophan pyrrolase activity by endotoxin may indicate an elevation of endogenous cortisone to a protective level before inhibitory amounts of endotoxin can be absorbed. The administration of excess cortisone results in a greater degree of induction in a 5° C environment. Endotoxin injected with cortisone under these conditions retards the rate of induction but not its eventuality. While the effect of endotoxin on tryptophan pyrrolase activity of mice in the cold is apparently different (19), the initial increase in activation of tryptophan pyrrolase is the same regardless of environmental temperature.

Preliminary experiments on the response of mice to endotoxin during heat stress suggest that in vivo tryptophan pyrrolase activity is depressed. In addition, this inhibition occurs sooner than in mice kept at 25° C.

The decrease in plasma iron concentration and in tryptophan pyrrolase activity of mice protected from the lethal effects of endotoxin by simultaneous

injections of ATP reflects again the inability of a protective compound to alter the course of the described metabolic changes associated with endointoxication. This suggests that the symptomology of endointoxication may yet be far removed from the actuality of events which either assure a prognosis of fatality or contribute to native resistance and pharmacologically mediated protection.

REFERENCES

1. Berry, L. J. and D. S. Smythe. "Effects of bacterial endotoxins on metabolism. VI. The role of tryptophan pyrrolase in response of mice to endotoxin." J. Exp. Med. 118:587-603, 1963.

2. Kampschmidt, R. F. and M. I. Arrendondo. "Some effects of endotoxin upon plasma iron turnover in the rat." Proc. Soc. Exp. Biol. Med. 113:142-145, 1963.

3. Eaves, G. N. and L. J. Berry. "Iron metabolism and tryptophan pyrrolase activity in endointoxicated mice." (Abstract) Fed. Proc. 23:563, 1964.

4. Previte, J. J. and L. J. Berry. "The effect of environmental temperature on the host-parasite relationship in mice." J. Infect. Dis. 110:201-209, 1962.

5. Landers, J. W. and B. Zak. "Determination of serum copper and iron in a single small sample." Amer. J. Clin. Path. 29:590-592, 1958.

6. Knox, W. E. and V. H. Auerbach. "The hormonal control of tryptophan peroxidase in the rat." J. Biol. Chem. 214:307 313, 1955.

7. Cartwright, G. E., M. A. Lauritsen, P. J. Jones, I. M. Merrill and M. M. Wintrobe. "The anemia of infection. I. Hypoferremia, hypercupremia, and alterations in porphyrin metabolism in patients." J. Clin. Invest. 25:65-80, 1946.

8. Wilcoxon, F. Some Rapid Approximate Statistical Procedures. New York, American Cyanamid Co., 1958.

9. Freireich, E. J., A. Miller, C. P. Emerson and J. F. Ross. "The effect of inflammation on the utilization of erythrocyte and transferrin iron for hemoglobin synthesis." J. Clin. Invest. 34:934P, 1955.

10. Kumar, S., S. Gupta and V. S. Mangalik. "Studies in anemia of infection. V. Role of reticulo-endothelial system in the regulation of plasma iron." Ind. J. M. Res. 47:388-392, 1959.

11. Cartwright, G. E., C. J. Gubler and M. M. Wintrobe. "The anemia of infection. XII. The effect of turpentine and colloidal thorium dioxide on the plasma iron and plasma copper of dogs." J. Biol. Chem. 184:579-587, 1960.

12. Heller, J. H. "Effect of cortisone on the function, capacity and activity of the reticulo-endothelial system." Fed. Proc. 12:65, 1953.

13. Biozzi, G., B. Benacerraf and B. N. Halpern. "The effect of Salm. typhi and its endotoxin on the phagocytic activity of the reticulo-endothelial system in mice." Brit. J. Exp. Path. 36:226-235, 1955.

14. Berry, L. J. Unpublished observation.

15. Schaefer, K. H. "The influence of infections and similar processes on the iron metabolism. III. The role of the reticulo-endothelial system, especially of the spleen, in the changes of iron metabolism influenced by infections." Z. ges. exptl. Med. 110:713-731, 1942. (In Chemical Abstracts 38:2384, 1944.)

16. Cartwright, G. E., M. A. Lauritsen, S. Humphreys, P. J. Jones, I. M. Merrill and M. M. Wintrobe. "The anemia of infection. II. The experimental production of hypoferremia and anemia in dogs." J. Clin. Invest. 25:81-86, 1946.

17. Kawachi, T. "Studies on liver tryptophan pyrrolase of tumor bearing rats." Kyushu J. Med. Sci. 13:285-298, 1962.

18. Feigelson, P. and O. Greengard. "The activation and induction of tryptophan pyrrolase during experimental porphyria and by amino-triazole." Biochim. Biophys. Acta 52:509-516, 1961.

19. Berry, L. J. "Endotoxin lethality and tryptophan pyrrolase induction in cold-exposed mice." Am. J. Physiol. (In press).

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
[redacted]
Bryn Mawr Coll[redacted]a

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
IRON METABOLISM AND TRYPTOPHAN PYRROLASE ACTIVITY IN ENDOTOXIN-POISONED AND CORTISONE-PROTECTED MICE.

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
[redacted] Quarterly Report, 1 Jun [redacted]64 - 31 Aug [redacted]64,

5. AUTHOR(S) (Last name, first name, initial)
Eaves, George N. and Berry, L. Joe

6. REPORT DATE
[redacted]

7a. TOTAL NO. OF PAGES
32

7b. NO. OF REFS
19

8a. [redacted] AF 41(609)-1764,

b. [redacted]8241,

c. [redacted]824101

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
None

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned
AAL-TR-64-27

10. AVAILABILITY/LIMITATION NOTICES
Qualified requesters may obtain copies of this report from DDC

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Arctic Aeromedical Laboratory
Fort Wainwright, Alaska

13. ABSTRACT The hypoferremia which follows the intraperitoneal injection of mice with heat-killed Salmonella typhimurium is not mitigated by protection with cortisone given simultaneously. A single injection of cortisone causes a transient hyperferremia. None of these changes in concentration of plasma iron occurs when mice are maintained in a 5° C environment during experimental treatment. The alteration in iron metabolism during endointoxication does not seem to affect the availability of the iron-porphyrin coenzyme of tryptophan pyrrolase. An initial response of mice to injections of endotoxin or cortisone or both is a translocation of hematin, and the availability of hematin permits an increase in tryptophan pyrrolase activity before the stress-mediated induction of the enzyme becomes apparent. The increase in available activator, with the subsequent decrease in amount of normally inactive apoenzyme, occurs at the same rate and to the same extent in mice housed at 5° C and 25° C. It is suggested that the increased demand for an iron-containing substance in the liver during stress contributes to the accompanying hypoferremia.

DD FORM 1 JAN 64 1473

UNCLASSIFIED

Security Classification

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
hypoferremia						
cortisone						
hyperferremia						
endointoxication						
hematin						
tryptophan pyrrolase						

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8a. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS), (S), (C), or (U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.

UNCLASSIFIED
Security Classification 4778-64